expr_otus_correlations

May 9, 2024

1 Correlations between transcriptome and OTU microbiome data

Notebook to run correlations between the host transcriptome data and OTUs from Dr. Wallace manuscript.

1.1 Datasets

1.1.1 RNAseq data

Matrix with TPM values from quantifying (Salmon) with cleaned reads against the maize transcriptome (representative transcripts of maize version 5)

1.1.2 16S data

The matrix that will be used corresponds to the OTU table generated by Dr. Wallace, which is in the FigShare repository lited in Wallace et al. (2018).

1.2 Association of identifiers between RNAseq (Kremling et al. 2018) and 16S datasets (Wallace et al. 2018)

File `0_kremling_expression_key.txt` from the publication FigShare has association of identifiers between samples. The NCBI SRA records have associations between the unique identifiers in each dataset (RNAseq or 16S) and the associations with the SRA unique identifiers.

[ ]:
```
#kremling_expression_key = '/media/renato/SSD1TB/Repositories/
 ↪maize_microbiome_transcriptomics/correlations_rnaseq_metataxonomics/
 ↪0_kremling_expression_key.txt'
#sra_run_table_16s = '/media/renato/SSD1TB/Repositories/
 ↪maize_microbiome_transcriptomics/16S_wallace2018/
 ↪SraRunInfo_Wallace_etal_2018.csv'
#sra_run_table_rnaseq = '/media/renato/SSD1TB/Repositories/
 ↪maize_microbiome_transcriptomics/rnaseq_kremling2018/run_info/
 ↪SraRunInfo_Kremling_etal_2018.csv'
kremling_expression_key = '/home/rsantos/Repositories/
 ↪maize_microbiome_transcriptomics/correlations_rnaseq_metataxonomics/
 ↪0_kremling_expression_key.txt'
sra_run_table_16s = '/home/rsantos/Repositories/
 ↪maize_microbiome_transcriptomics/16S_wallace2018/
 ↪SraRunInfo_Wallace_etal_2018.csv'
```

```
sra_run_table_rnaseq = '/home/rsantos/Repositories/
 ↪maize_microbiome_transcriptomics/rnaseq_kremling2018/run_info/
 ↪SraRunInfo_Kremling_etal_2018.csv'

dict_wallace_kremling_2018 = {}
kremling_expression_key_dict = {}
```

[ ]:
```
with open(kremling_expression_key, 'r') as file:

    _ = file.readline()

    for line in file:
        fields = line.strip().split('\t')

        kremling_identifier = fields[0]
        wallace_identifier = fields[1]

        # Adding both identifiers (Wallace and Kremling) to the dictionary
        kremling_expression_key_dict[kremling_identifier] = wallace_identifier
        kremling_expression_key_dict[wallace_identifier] = kremling_identifier
```

[ ]:
```
import re

with open(sra_run_table_rnaseq, 'r') as file:

    _ = file.readline()

    for line in file:
        fields = line.strip().split(',')
        fields2 = fields[11].split('_')
        rnaseq_run_id = fields[0]
        sample_id = fields2[1]
        rnaseq_genotype = fields2[2]
        day = ''
        match = re.search(r'\d+', sample_id)
        unmatched_parts = re.split(r'\d+', sample_id)
        day_period = unmatched_parts[0]
        if match:
            day = int(match.group())
        if sample_id.startswith('LMA') and rnaseq_genotype != '#N/A':
            dict_wallace_kremling_2018[fields[11]] = {'run_accession_16s': '',
                                    'run_accession_rnaseq': rnaseq_run_id,
                                    'day': day,
                                    'day_period': day_period,
                                    'genotype_16s': '',
                                    'genotype_rnaseq': rnaseq_genotype}
```

[ ]:
```
rnaseq_samples_with_16s = 0

with open(sra_run_table_16s, 'r') as file:

    _ = file.readline()

    for line in file:
        fields = line.strip().split(',')
        fields2 = fields[11].split('.')
        metataxonomics_run_id = fields[0]
        day = int(fields2[1])
        day_period = fields2[0]
        for key, value in kremling_expression_key_dict.items():
            if value == fields[11]:
                if dict_wallace_kremling_2018[key]['day'] != day:
                    print('Big problem!')
                    print(day, dict_wallace_kremling_2018[key]['day'])
                    print(dict_wallace_kremling_2018[key])
                    print(value, fields[11], key)
                    exit(1)
                if dict_wallace_kremling_2018[key]['day_period'] != day_period:
                    if key == '10343927_LMAN8_CML505_CAACAG':
                        continue
                    else:
                        print(day_period,␣
 ↪dict_wallace_kremling_2018[key]['day_period'])
                        print(dict_wallace_kremling_2018[key])
                        print(value, fields[11], key)
                        exit(1)
                dict_wallace_kremling_2018[key]['run_accession_16s'] =␣
 ↪metataxonomics_run_id
                rnaseq_samples_with_16s+=1

print(f'{rnaseq_samples_with_16s} sample pairs found.')
```

```
484 sample pairs found.
```

[ ]:
```
no_16s = 0
for key, value in dict_wallace_kremling_2018.items():
    if value['run_accession_16s'] == '':
        print(key, value)
        no_16s+=1
print(f'{no_16s} samples without 16S data.')
```

```
10343927_LMAN8_B73_CACACT {'run_accession_16s': '', 'run_accession_rnaseq':
'SRR5909633', 'day': 8, 'day_period': 'LMAN', 'genotype_16s': '',
'genotype_rnaseq': 'B73'}
```

```
10343927_LMAN8_CML505_CAACAG {'run_accession_16s': '', 'run_accession_rnaseq':
'SRR5911345', 'day': 8, 'day_period': 'LMAN', 'genotype_16s': '',
'genotype_rnaseq': 'CML505'}
2 samples without 16S data.
```

2 Generating a matrix with both RNAseq and OTUs from Wallace et al (2018)

Associations between 16S and RNAseq data are present in the 'dict_wallace_kremling_2018' dictionary.

[ ]:
```
run2my_sample_id = {}

for key in dict_wallace_kremling_2018:
    if dict_wallace_kremling_2018[key]['run_accession_rnaseq']:
        ␣
 ↪run2my_sample_id[dict_wallace_kremling_2018[key]['run_accession_rnaseq']] =␣
 ↪key
    if dict_wallace_kremling_2018[key]['run_accession_16s']:
        run2my_sample_id[dict_wallace_kremling_2018[key]['run_accession_16s']]␣
 ↪= key
```

[ ]:
```
import pandas as pd

# Importing expression data from Kremling et al. 2018 (TPM matrix on Maize v5␣
 ↪using Salmon after cleaning with TrimGalore)
#kremling_expression_v5 = pd.read_csv('/media/renato/SSD1TB/Projects/UGA_RACS/
 ↪RNAseq/Salmon/Zma2_tpm_matrix.txt', sep='\t')
kremling_expression_v5 = pd.read_csv('/media/rsantos/4TB_drive/Projects/
 ↪UGA_RACS/RNAseq/Salmon/Zma2_tpm_matrix.txt', sep='\t')

# Rename column and reset the index
kremling_expression_v5.set_index('Name', inplace=True)

# Print the dataframe
kremling_expression_v5.head()
```

[ ]:

Name	SRR5909626	SRR5909627	SRR5909633	SRR5909635	\
Zm00001eb371370_T002	1.04145	0.0000	3.391060	0.0	
Zm00001eb371350_T001	0.00000	0.0000	0.000000	0.0	
Zm00001eb371330_T001	0.00000	0.0000	0.000000	0.0	
Zm00001eb371310_T001	0.00000	0.0000	0.000000	0.0	
Zm00001eb371280_T001	1.27650	2.1092	0.692731	0.0	

Name	SRR5909639	SRR5909642	SRR5909645	SRR5909653	\

```
Zm00001eb371370_T002      0.0000     1.82712    0.284514     2.23201
Zm00001eb371350_T001      0.0000     0.00000    0.000000     0.00000
Zm00001eb371330_T001      0.0000     0.00000    0.000000     0.00000
Zm00001eb371310_T001      0.0000     0.00000    0.000000     0.00000
Zm00001eb371280_T001      4.2798     1.47496    2.557320     0.00000

                     SRR5909655  SRR5909665  …  SRR5912073  SRR5912081  \
Name                                         …
Zm00001eb371370_T002   0.437147    0.468934  …     0.00000     1.51042
Zm00001eb371350_T001   0.000000    0.000000  …     0.00000     0.00000
Zm00001eb371330_T001   0.000000    0.000000  …     0.00000     0.00000
Zm00001eb371310_T001   0.000000    0.000000  …     0.00000     0.00000
Zm00001eb371280_T001   1.065940    1.149530  …     3.02253     0.41140

                     SRR5912082  SRR5912083  SRR5912093  SRR5912094  \
Name
Zm00001eb371370_T002     0.00000         0.0     0.00000     2.82055
Zm00001eb371350_T001     0.00000         0.0     0.00000     0.00000
Zm00001eb371330_T001     0.00000         0.0     0.00000     0.00000
Zm00001eb371310_T001     0.00000         0.0     0.00000     0.00000
Zm00001eb371280_T001     1.17447         0.0     3.48749     9.47506

                     SRR5912104  SRR5912105  SRR5912111  SRR5912116
Name
Zm00001eb371370_T002     3.96967     0.00000     2.96105     0.00000
Zm00001eb371350_T001     0.00000     0.00000     0.00000     0.00000
Zm00001eb371330_T001     0.00000     0.00000     0.00000     0.00000
Zm00001eb371310_T001     0.00000     0.00000     0.00000     0.00000
Zm00001eb371280_T001     6.19189     3.80776     1.03695     1.14981

[5 rows x 486 columns]
```

Renaming columns Renaming columns of Kremling data based on associations in 'run2my_sample_id':

[ ]:
```
kremling_expression_v5 = kremling_expression_v5.rename(columns=run2my_sample_id)
kremling_expression_v5.columns = [str(x) for x in kremling_expression_v5.
  ↪columns]

kremling_expression_v5.head()
```

[ ]:
```
                     10343927_LMAD26_CI21E_AAGTGG  \
Name
Zm00001eb371370_T002                       1.04145
Zm00001eb371350_T001                       0.00000
Zm00001eb371330_T001                       0.00000
Zm00001eb371310_T001                       0.00000
```

Zm00001eb371280_T001	1.27650

Name	10343264_LMAN26_CI21E_ATGAAC	10343927_LMAN8_B73_CACACT	\
Zm00001eb371370_T002	0.0000	3.391060	
Zm00001eb371350_T001	0.0000	0.000000	
Zm00001eb371330_T001	0.0000	0.000000	
Zm00001eb371310_T001	0.0000	0.000000	
Zm00001eb371280_T001	2.1092	0.692731	

Name	10343264_LMAN26_B64_ACCAGT	10343262_LMAN8_B109_TGCTAT	\
Zm00001eb371370_T002	0.0	0.0000	
Zm00001eb371350_T001	0.0	0.0000	
Zm00001eb371330_T001	0.0	0.0000	
Zm00001eb371310_T001	0.0	0.0000	
Zm00001eb371280_T001	0.0	4.2798	

Name	10343262_LMAN8_B14A_CTCTCG	10343262_LMAN8_B57_CCTAAG	\
Zm00001eb371370_T002	1.82712	0.284514	
Zm00001eb371350_T001	0.00000	0.000000	
Zm00001eb371330_T001	0.00000	0.000000	
Zm00001eb371310_T001	0.00000	0.000000	
Zm00001eb371280_T001	1.47496	2.557320	

Name	10343927_LMAD26_B77_TAATCG	10343262_LMAN8_B79_GCAGCC	\
Zm00001eb371370_T002	2.23201	0.437147	
Zm00001eb371350_T001	0.00000	0.000000	
Zm00001eb371330_T001	0.00000	0.000000	
Zm00001eb371310_T001	0.00000	0.000000	
Zm00001eb371280_T001	0.00000	1.065940	

Name	10343927_LMAN8_CI187-2_GACGAT	…	\
		…	
Zm00001eb371370_T002	0.468934	…	
Zm00001eb371350_T001	0.000000	…	
Zm00001eb371330_T001	0.000000	…	
Zm00001eb371310_T001	0.000000	…	
Zm00001eb371280_T001	1.149530	…	

Name	10344826_LMAN8_I29_ACGTCT	10344823_LMAD8_IA2132_ACACGC	\
Zm00001eb371370_T002	0.00000	1.51042	
Zm00001eb371350_T001	0.00000	0.00000	
Zm00001eb371330_T001	0.00000	0.00000	

Zm00001eb371310_T001	0.00000	0.00000
Zm00001eb371280_T001	3.02253	0.41140

Name	10343264_LMAD26_CML91_AACGCC \
Zm00001eb371370_T002	0.00000
Zm00001eb371350_T001	0.00000
Zm00001eb371330_T001	0.00000
Zm00001eb371310_T001	0.00000
Zm00001eb371280_T001	1.17447

Name	10344827_LMAN26_CML91_AATCCG \
Zm00001eb371370_T002	0.0
Zm00001eb371350_T001	0.0
Zm00001eb371330_T001	0.0
Zm00001eb371310_T001	0.0
Zm00001eb371280_T001	0.0

Name	10344827_LMAN26_Ki21_AAGACA \
Zm00001eb371370_T002	0.00000
Zm00001eb371350_T001	0.00000
Zm00001eb371330_T001	0.00000
Zm00001eb371310_T001	0.00000
Zm00001eb371280_T001	3.48749

Name	10343927_LMAD26_Ki21_ACGTCT \
Zm00001eb371370_T002	2.82055
Zm00001eb371350_T001	0.00000
Zm00001eb371330_T001	0.00000
Zm00001eb371310_T001	0.00000
Zm00001eb371280_T001	9.47506

Name	10344826_LMAD8_E2558W_CGCAAC \
Zm00001eb371370_T002	3.96967
Zm00001eb371350_T001	0.00000
Zm00001eb371330_T001	0.00000
Zm00001eb371310_T001	0.00000
Zm00001eb371280_T001	6.19189

Name	10343927_LMAN8_E2558W_GAACCT \
Zm00001eb371370_T002	0.00000
Zm00001eb371350_T001	0.00000

```
Zm00001eb371330_T001                        0.00000
Zm00001eb371310_T001                        0.00000
Zm00001eb371280_T001                        3.80776

                     10344826_LMAD8_IDS69_CAGGAC  10343927_LMAN8_IDS69_ACATTA
Name
Zm00001eb371370_T002                      2.96105                      0.00000
Zm00001eb371350_T001                      0.00000                      0.00000
Zm00001eb371330_T001                      0.00000                      0.00000
Zm00001eb371310_T001                      0.00000                      0.00000
Zm00001eb371280_T001                      1.03695                      1.14981

[5 rows x 486 columns]
```

Testing correlations between D and N samples for RNAseq Running correlations between all pairs of RNAseq samples - N and D - and generate a histogram of the values.

[ ]:
```
import numpy as np

rnaseq_pairs_cors = []
rnaseq_pairs2correlate = {}
ones = 0
twos = 0
threes_or_more = 0

for key in dict_wallace_kremling_2018.keys():
    if␣
 ↪str(dict_wallace_kremling_2018[key]['day'])+'_'+dict_wallace_kremling_2018[key]['genotype_r
 ↪not in rnaseq_pairs2correlate.keys():
        ␣
 ↪rnaseq_pairs2correlate[str(dict_wallace_kremling_2018[key]['day'])+'_'+dict_wallace_kremlin
    else:
        ␣
 ↪rnaseq_pairs2correlate[str(dict_wallace_kremling_2018[key]['day'])+'_'+dict_wallace_kremlin
 ↪append(key)

for key in rnaseq_pairs2correlate.keys():
    if len(rnaseq_pairs2correlate[key]) == 1:
        ones+=1
    elif len(rnaseq_pairs2correlate[key]) == 2:
        twos+=1
        column1 = kremling_expression_v5[rnaseq_pairs2correlate[key][0]]
        column2 = kremling_expression_v5[rnaseq_pairs2correlate[key][1]]
        correlation = np.corrcoef(column1, column2)[0, 1]
        rnaseq_pairs_cors.append(correlation)
    elif len(rnaseq_pairs2correlate[key]) >= 3:
```

```
        threes_or_more+=1
    else:
        continue

print(f'cases with ones: {ones}')
print(f'cases with twos: {twos}')
print(f'cases with threes: {threes_or_more}')
```

```
cases with ones: 60
cases with twos: 203
cases with threes: 2
```

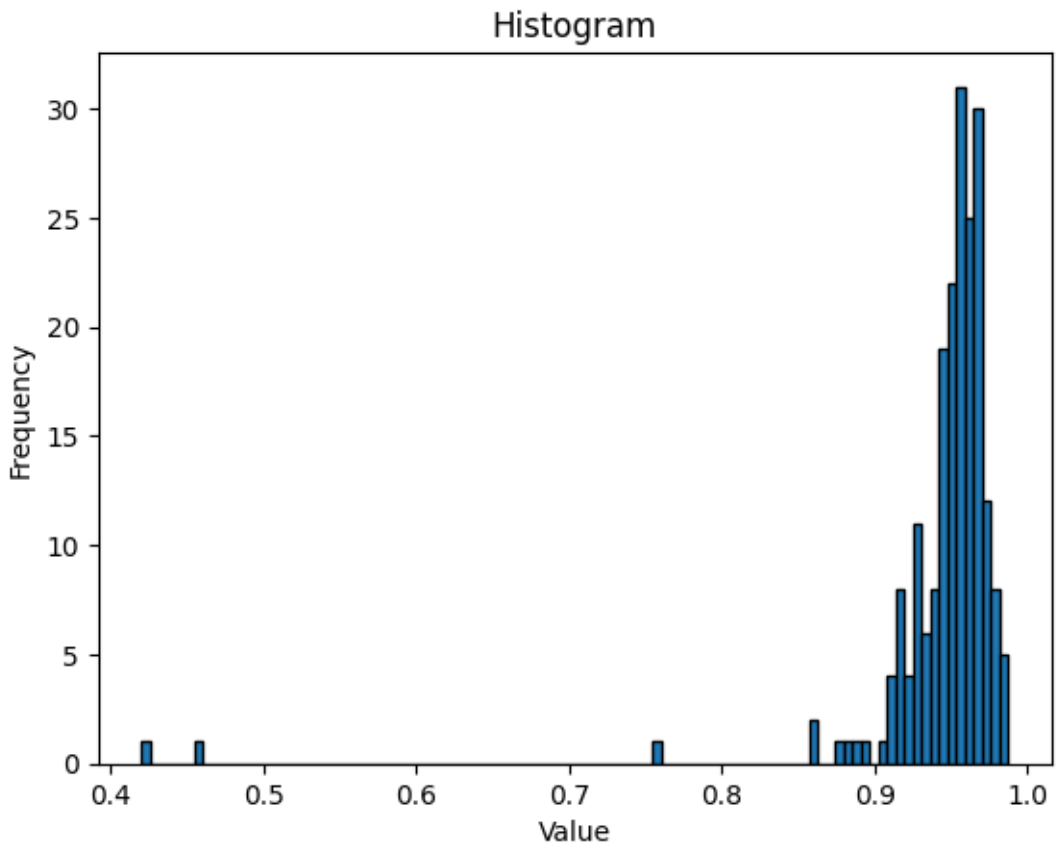
There are 203 pairs (day and night) of samples in the RNAseq data available for the correlation analyses. I (RACS) used these pairs to see an overall distribution of the correlation values (how day and night samples correlate with each other).

[ ]:
```
import matplotlib.pyplot as plt

# Build histogram
plt.hist(rnaseq_pairs_cors, bins=100, edgecolor='black')

# Add labels and title
plt.xlabel('Value')
plt.ylabel('Frequency')
plt.title('Histogram')

# Show the plot
plt.show()
```



It looks like there is a high correlation in most of the pairs (D and N for same genes).

However, we (RACS and JW) decided to run correlations between OTUs and RNAseq for days and night, independently, to see which correlations are common and specific to each day period.

Importing OTU data OTU table was obtained from the FigShare repo associated with Wallace et al. (2018), with the `.biom` extension. It was converted to a tabular file as described in the Gitlab wiki associated with this project (first line of hashtag was removed and 'OTU ID' column was renamed to 'OTU'). Since the taxonomy is not important at this initial moment, the taxonomy column was also removed from the table. Next, day and nigth samples were merged for the OTU table.

Importing OTU data with merged day and night samples OTU counts for day and night samples were merged in a separate notebook and imported:

[ ]:
```
#otu_table_merged_d_n = pd.read_csv('/media/renato/SSD1TB/Repositories/
 ↪maize_microbiome_transcriptomics/16S_wallace2018/combine_day_night_samples/
 ↪summed_day_night_otu_counts.tsv',
#                                    sep='\t', index_col='OTU ID')
```

```
otu_table_merged_d_n = pd.read_csv('/home/rsantos/Repositories/
 ↪maize_microbiome_transcriptomics/16S_wallace2018/combine_day_night_samples/
 ↪summed_day_night_otu_counts.tsv',
                                    sep='\t', index_col='OTU ID')

otu_table_merged_d_n.head()
```

[ ]:

OTU ID	14A0247_8	14A0051_8	14A0381_26	14A0533_26	14A0281_8	14A0295_8	\
4479944	1.0	2.0	3.0	1.0	0.0	0.0	
995900	0.0	0.0	0.0	0.0	5.0	8.0	
1124709	0.0	0.0	0.0	0.0	0.0	0.0	
541139	0.0	0.0	0.0	0.0	0.0	0.0	
533625	0.0	1.0	2.0	0.0	0.0	0.0	

OTU ID	14A0169_26	14A0069_8	14A0497_26	14A0023_8	…	14A0345_8	\
					…		
4479944	0.0	0.0	0.0	0.0	…	0.0	
995900	6.0	1.0	1.0	1.0	…	0.0	
1124709	0.0	0.0	0.0	0.0	…	0.0	
541139	0.0	0.0	0.0	0.0	…	0.0	
533625	0.0	0.0	40.0	1.0	…	0.0	

OTU ID	14A0267_8	14A0009_8	14A0007_8	14A0093_26	14A0137_26	14A0265_8	\
4479944	0.0	0.0	0.0	0.0	0.0	0.0	
995900	0.0	0.0	0.0	0.0	0.0	0.0	
1124709	0.0	0.0	0.0	0.0	0.0	0.0	
541139	0.0	0.0	0.0	0.0	0.0	0.0	
533625	0.0	0.0	1.0	0.0	0.0	0.0	

OTU ID	14A0155_26	14A0167_26	14A0481_26
4479944	0.0	0.0	0.0
995900	0.0	0.0	0.0
1124709	0.0	0.0	0.0
541139	0.0	0.0	0.0
533625	2.0	0.0	2.0

[5 rows x 249 columns]

Creating a dictionary that will be used later to rename columns of the RNAseq matrix (to match 16S columns):

[ ]:
```
kremling2merged_d_n = {}

for key, value in rnaseq_pairs2correlate.items():
```

```
    if len(rnaseq_pairs2correlate[key]) == 2:
        for rnaseq_id in rnaseq_pairs2correlate[key]:
            fields = kremling_expression_key_dict[rnaseq_id].split('.')
            kremling2merged_d_n[rnaseq_id] = fields[2]+'_'+str(fields[1])
```

Selecting Day and Night samples separately for the RNAseq For creating the dataframes with day and night RNAseq samples, I (RACS):

- Ensured to select samples that had pairs (day and night samples were available)
- Ensured there was a corresponding set of microbiome samples for the same set of RNAseq

[ ]:
```
kremling2_day_samples_dict = {}
kremling2_night_samples_dict = {}
problematic_samples = []

for key, value in rnaseq_pairs2correlate.items():
    if len(rnaseq_pairs2correlate[key]) == 2:
        n_count = 0
        d_count = 0
        for plot_day_id in rnaseq_pairs2correlate[key]:
            fields = kremling_expression_key_dict[plot_day_id].split('.')
            if fields[0] == 'LMAD' and d_count == 0:
                kremling2_day_samples_dict[plot_day_id] =␣
 ↪fields[2]+'_'+str(fields[1])
                d_count+=1
            elif fields[0] == 'LMAN' and n_count == 0:
                kremling2_night_samples_dict[plot_day_id] =␣
 ↪fields[2]+'_'+str(fields[1])
                n_count+=1
            else:
                problematic_samples.append(rnaseq_pairs2correlate[key])

print(f'There are {len(kremling2_day_samples_dict)} day samples')
print(f'There are {len(kremling2_night_samples_dict)} night samples')
```

```
There are 203 day samples
There are 202 night samples
```

[ ]:
```
kremling_expression_v5_day = kremling_expression_v5.
 ↪filter(items=list(kremling2_day_samples_dict.keys()))
print(kremling_expression_v5_day.shape)
print(kremling_expression_v5_day.head(n=2))
kremling_expression_v5_night = kremling_expression_v5.
 ↪filter(items=list(kremling2_night_samples_dict.keys()))
print(kremling_expression_v5_night.shape)
print(kremling_expression_v5_night.head(n=2))
```

```
(39096, 203)
                    10343927_LMAD26_CI21E_AAGTGG  \
Name
Zm00001eb371370_T002                       1.04145
Zm00001eb371350_T001                       0.00000

                    10343927_LMAD26_B64_GCGAAT  10343927_LMAD26_B77_TAATCG  \
Name
Zm00001eb371370_T002                    0.450365                     2.23201
Zm00001eb371350_T001                    0.000000                     0.00000

                    10344823_LMAD8_A661_GTCAGG  10344823_LMAD8_A679_TAGGCT  \
Name
Zm00001eb371370_T002                     3.43127                     2.25646
Zm00001eb371350_T001                     0.00000                     0.00000

                    10343927_LMAD26_4226_TGGCGA  \
Name
Zm00001eb371370_T002                    0.529255
Zm00001eb371350_T001                    0.000000

                    10343264_LMAD26_CI66_CAGATG  \
Name
Zm00001eb371370_T002                      2.9897
Zm00001eb371350_T001                      0.0000

                    10344823_LMAD8_CM105_ACCAGT  \
Name
Zm00001eb371370_T002                     2.93577
Zm00001eb371350_T001                     0.00000

                    10344823_LMAD8_CH701-30_ATGAAC  \
Name
Zm00001eb371370_T002                        3.08344
Zm00001eb371350_T001                        0.00000

                    10344823_LMAD8_C49A_AATGAA  …  \
Name                                            …
Zm00001eb371370_T002                    3.37505  …
Zm00001eb371350_T001                    0.00000  …

                    10344823_LMAD8_CO255_AATAGC  \
Name
Zm00001eb371370_T002                    0.375458
Zm00001eb371350_T001                    0.000000

                    10344823_LMAD8_CO125_CTCCAT  \
Name
```

```
Zm00001eb371370_T002                      1.56496
Zm00001eb371350_T001                      0.00000

                     10343264_LMAD26_CML228_CCGCAA  \
Name
Zm00001eb371370_T002                        0.89365
Zm00001eb371350_T001                        0.00000

                     10343927_LMAD26_K64_CCTGCT  \
Name
Zm00001eb371370_T002                         0.0
Zm00001eb371350_T001                         0.0

                     10343264_LMAD26_CML323_GCAGCC  \
Name
Zm00001eb371370_T002                        1.72298
Zm00001eb371350_T001                        0.00000

                     10344823_LMAD8_I29_ACAGAT  10343264_LMAD26_CML91_AACGCC  \
Name
Zm00001eb371370_T002                    1.07412                           0.0
Zm00001eb371350_T001                    0.00000                           0.0

                     10343927_LMAD26_Ki21_ACGTCT  \
Name
Zm00001eb371370_T002                      2.82055
Zm00001eb371350_T001                      0.00000

                     10344826_LMAD8_E2558W_CGCAAC  \
Name
Zm00001eb371370_T002                       3.96967
Zm00001eb371350_T001                       0.00000

                     10344826_LMAD8_IDS69_CAGGAC
Name
Zm00001eb371370_T002                      2.96105
Zm00001eb371350_T001                      0.00000

[2 rows x 203 columns]
(39096, 202)
                     10343264_LMAN26_CI21E_ATGAAC  \
Name
Zm00001eb371370_T002                          0.0
Zm00001eb371350_T001                          0.0

                     10343264_LMAN26_B64_ACCAGT  10343264_LMAN26_B77_GTAGAA  \
Name
Zm00001eb371370_T002                         0.0                         0.0
```

```
Zm00001eb371350_T001                          0.0                          0.0

                    10344826_LMAN8_A661_ACCGTG  10344826_LMAN8_A679_ATTGGT  \
Name
Zm00001eb371370_T002                          0.0                     0.451347
Zm00001eb371350_T001                          0.0                     0.000000

                    10343264_LMAN26_4226_TGTGCA  \
Name
Zm00001eb371370_T002                           0.0
Zm00001eb371350_T001                           0.0

                    10344827_LMAN26_CI66_GTGTAG  \
Name
Zm00001eb371370_T002                     0.235701
Zm00001eb371350_T001                     0.000000

                    10344826_LMAN8_CM105_GATTGT  \
Name
Zm00001eb371370_T002                           0.0
Zm00001eb371350_T001                           0.0

                    10343262_LMAN8_CH701-30_GAACCT  \
Name
Zm00001eb371370_T002                              0.0
Zm00001eb371350_T001                              0.0

                    10344826_LMAN8_C49A_GGTATA  …  \
Name                                            …
Zm00001eb371370_T002                          0.0  …
Zm00001eb371350_T001                          0.0  …

                    10344826_LMAN8_CO255_CTCATA  \
Name
Zm00001eb371370_T002                           0.0
Zm00001eb371350_T001                           0.0

                    10344826_LMAN8_CO125_TGTGCA  \
Name
Zm00001eb371370_T002                           0.0
Zm00001eb371350_T001                           0.0

                    KAKRNA14_LMAN26_CML228_ACATTA  \
Name
Zm00001eb371370_T002                         1.75355
Zm00001eb371350_T001                         0.00000

                    10344827_LMAN26_K64_ACCTAC  \
```

```
Name
Zm00001eb371370_T002                    0.465999
Zm00001eb371350_T001                    0.000000

                     10344827_LMAN26_CML323_GTGCCA  \
Name
Zm00001eb371370_T002                       0.283385
Zm00001eb371350_T001                       0.000000

                     10344826_LMAN8_I29_ACGTCT  10344827_LMAN26_CML91_AATCCG  \
Name
Zm00001eb371370_T002                        0.0                           0.0
Zm00001eb371350_T001                        0.0                           0.0

                     10344827_LMAN26_Ki21_AAGACA  \
Name
Zm00001eb371370_T002                         0.0
Zm00001eb371350_T001                         0.0

                     10343927_LMAN8_E2558W_GAACCT  \
Name
Zm00001eb371370_T002                          0.0
Zm00001eb371350_T001                          0.0

                     10343927_LMAN8_IDS69_ACATTA
Name
Zm00001eb371370_T002                         0.0
Zm00001eb371350_T001                         0.0

[2 rows x 202 columns]
```

[ ]:
```
kremling_expression_v5_day_renamed = kremling_expression_v5_day.
  ↪rename(columns=kremling2merged_d_n)
print(kremling_expression_v5_day_renamed.head())
kremling_expression_v5_night_renamed = kremling_expression_v5_night.
  ↪rename(columns=kremling2merged_d_n)
print(kremling_expression_v5_night_renamed.head())
```

```
                     14A0253_26  14A0041_26  14A0171_26  14A0045_8  \
Name
Zm00001eb371370_T002     1.04145    0.450365     2.23201    3.43127
Zm00001eb371350_T001     0.00000    0.000000     0.00000    0.00000
Zm00001eb371330_T001     0.00000    0.000000     0.00000    0.00000
Zm00001eb371310_T001     0.00000    0.000000     0.00000    0.00000
Zm00001eb371280_T001     1.27650    0.736011     0.00000    2.40324

                     14A0085_8  14A0079_26  14A0467_26  14A0039_8  14A0095_8  \
Name
```

```
Zm00001eb371370_T002    2.25646    0.529255     2.98970    2.93577    3.08344
Zm00001eb371350_T001    0.00000    0.000000     0.00000    0.00000    0.00000
Zm00001eb371330_T001    0.00000    0.000000     0.00000    0.00000    0.00000
Zm00001eb371310_T001    0.00000    0.000000     0.00000    0.00000    0.00000
Zm00001eb371280_T001    5.79484    1.297400     2.74828    3.42699    5.03912

                    14A0037_8  …  14A0005_8  14A0027_8  14A0533_26  \
Name                           …
Zm00001eb371370_T002    3.37505  …   0.375458    1.56496    0.893650
Zm00001eb371350_T001    0.00000  …   0.000000    0.00000    0.000000
Zm00001eb371330_T001    0.00000  …   0.000000    0.00000    0.000000
Zm00001eb371310_T001    0.00000  …   0.000000    0.00000    0.406263
Zm00001eb371280_T001    1.48499  …   3.374760    8.95139    0.365113

                    14A0333_26  14A0473_26  14A0047_8  14A0453_26  \
Name
Zm00001eb371370_T002     0.00000    1.722980    1.07412     0.00000
Zm00001eb371350_T001     0.00000    0.000000    0.00000     0.00000
Zm00001eb371330_T001     0.00000    0.000000    0.00000     0.00000
Zm00001eb371310_T001     0.00000    0.000000    0.00000     0.00000
Zm00001eb371280_T001     4.15375    0.703948    5.26615     1.17447

                    14A0367_26  14A0345_8  14A0343_8
Name
Zm00001eb371370_T002     2.82055    3.96967    2.96105
Zm00001eb371350_T001     0.00000    0.00000    0.00000
Zm00001eb371330_T001     0.00000    0.00000    0.00000
Zm00001eb371310_T001     0.00000    0.00000    0.00000
Zm00001eb371280_T001     9.47506    6.19189    1.03695

[5 rows x 203 columns]
                    14A0253_26  14A0041_26  14A0171_26  14A0045_8  \
Name
Zm00001eb371370_T002      0.0000         0.0    0.000000   0.000000
Zm00001eb371350_T001      0.0000         0.0    0.000000   0.000000
Zm00001eb371330_T001      0.0000         0.0    0.000000   0.000000
Zm00001eb371310_T001      0.0000         0.0    0.000000   0.000000
Zm00001eb371280_T001      2.1092         0.0    0.517418   0.492978

                    14A0085_8  14A0079_26  14A0467_26  14A0039_8  14A0095_8  \
Name
Zm00001eb371370_T002   0.451347    0.000000    0.235701    0.00000        0.0
Zm00001eb371350_T001   0.000000    0.000000    0.000000    0.00000        0.0
Zm00001eb371330_T001   0.000000    0.000000    0.000000    0.00000        0.0
Zm00001eb371310_T001   0.000000    0.000000    0.000000    0.00000        0.0
Zm00001eb371280_T001   1.844030    0.502906    1.348180    2.12836        0.0

                    14A0037_8  …  14A0005_8  14A0027_8  14A0533_26  \
```

```
Name                                 …
Zm00001eb371370_T002    0.00000  …        0.0    0.00000     1.75355
Zm00001eb371350_T001    0.00000  …        0.0    0.00000     0.00000
Zm00001eb371330_T001    0.00000  …        0.0    0.00000     0.00000
Zm00001eb371310_T001    0.00000  …        0.0    0.00000     0.00000
Zm00001eb371280_T001    1.36407  …        0.0    2.84774     0.00000

                     14A0333_26  14A0473_26  14A0047_8  14A0453_26  \
Name
Zm00001eb371370_T002    0.465999    0.283385    0.00000         0.0
Zm00001eb371350_T001    0.000000    0.000000    0.00000         0.0
Zm00001eb371330_T001    0.000000    0.000000    0.00000         0.0
Zm00001eb371310_T001    0.000000    0.000000    0.00000         0.0
Zm00001eb371280_T001    1.523120    0.231562    3.02253         0.0

                     14A0367_26  14A0345_8  14A0343_8
Name
Zm00001eb371370_T002     0.00000    0.00000    0.00000
Zm00001eb371350_T001     0.00000    0.00000    0.00000
Zm00001eb371330_T001     0.00000    0.00000    0.00000
Zm00001eb371310_T001     0.00000    0.00000    0.00000
Zm00001eb371280_T001     3.48749    3.80776    1.14981

[5 rows x 202 columns]
```

[ ]:
```
otu_table_merged_day = otu_table_merged_d_n.
  ↪filter(items=list(kremling2_day_samples_dict.values()))
print(otu_table_merged_day.shape)
otu_table_merged_night = otu_table_merged_d_n.
  ↪filter(items=list(kremling2_night_samples_dict.values()))
print(otu_table_merged_night.shape)
```

```
(9057, 173)
(9057, 172)
```

[ ]:
```
kremling_expression_v5_day_renamed_filtered =␣
  ↪kremling_expression_v5_day_renamed.filter(items=otu_table_merged_day.columns)
print(kremling_expression_v5_day_renamed_filtered.shape)
kremling_expression_v5_night_renamed_filtered =␣
  ↪kremling_expression_v5_night_renamed.filter(items=otu_table_merged_night.
  ↪columns)
print(kremling_expression_v5_night_renamed_filtered.shape)
```

```
(39096, 173)
(39096, 172)
```

[ ]:
```
if kremling_expression_v5_day_renamed_filtered.columns.all() ==␣
 ↪otu_table_merged_day.columns.all():
    print('Columns are equal (day)!')
if kremling_expression_v5_night_renamed_filtered.columns.all() ==␣
 ↪otu_table_merged_night.columns.all():
    print('Columns are equal (night)!')
```

```
Columns are equal (day)!
Columns are equal (night)!
```

[ ]:
```
def count_zeros(df, threshold=0.5):
    # Count the number of zeros in each row
    # If the number of zeros is greater than the threshold, remove the row
    threshold_int = int(df.shape[1] * threshold)
    print(f'Threshold: {threshold_int} (threshold * number of columns)')
    zero_counts = df.apply(lambda row: (row == 0).sum(), axis=1)
    return df[zero_counts < threshold_int]
```

[ ]:
```
import pandas as pd

otu_table_merged_day_cpm = otu_table_merged_day.divide(otu_table_merged_day.
 ↪sum())
otu_table_merged_day_cpm = otu_table_merged_day_cpm * 1e6
otu_table_merged_night_cpm = otu_table_merged_night.
 ↪divide(otu_table_merged_night.sum())
otu_table_merged_night_cpm = otu_table_merged_night_cpm * 1e6
```

[ ]:
```
otu_table_merged_day_zeros_filtered = count_zeros(otu_table_merged_day_cpm)
otu_table_merged_night_zeros_filtered = count_zeros(otu_table_merged_night_cpm)
kremling_expression_v5_day_renamed_zeros_filtered =␣
 ↪count_zeros(kremling_expression_v5_day_renamed_filtered)
kremling_expression_v5_night_renamed_zeros_filtered =␣
 ↪count_zeros(kremling_expression_v5_night_renamed_filtered)
print(otu_table_merged_day_zeros_filtered.shape)
print(otu_table_merged_night_zeros_filtered.shape)
print(kremling_expression_v5_day_renamed_zeros_filtered.shape)
print(kremling_expression_v5_night_renamed_zeros_filtered.shape)
```

```
Threshold: 86 (threshold * number of columns)
Threshold: 86 (threshold * number of columns)
Threshold: 86 (threshold * number of columns)
Threshold: 86 (threshold * number of columns)
(1117, 173)
(1135, 172)
(19692, 173)
(20049, 172)
```

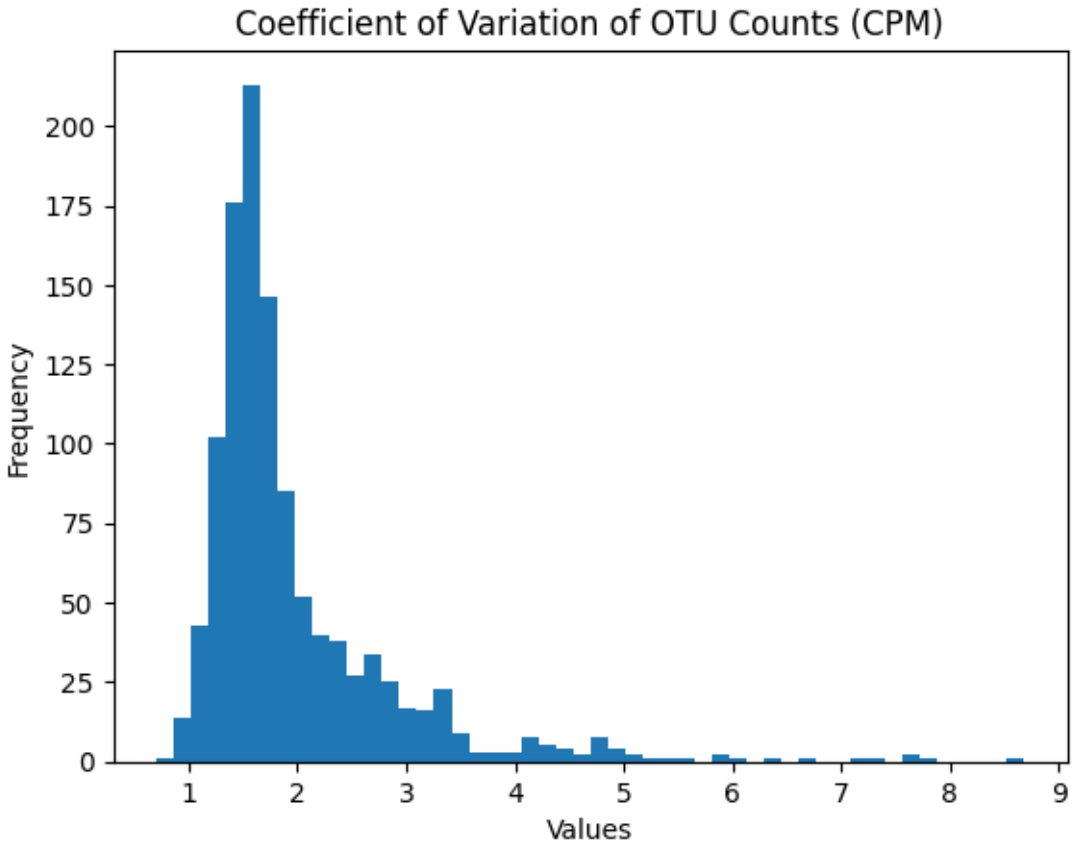
[ ]:
```
# Calculate the coefficient of variation for each row
otu_table_merged_day_zeros_filtered_cv = otu_table_merged_day_zeros_filtered.
 ↪apply(lambda row: np.std(row) / np.mean(row), axis=1)
otu_table_merged_night_zeros_filtered_cv =␣
 ↪otu_table_merged_night_zeros_filtered.apply(lambda row: np.std(row) / np.
 ↪mean(row), axis=1)
```

[ ]:
```
import matplotlib.pyplot as plt

otu_table_merged_day_zeros_filtered_cv.plot.hist(bins=50)

# Set the title and labels
plt.title("Coefficient of Variation of OTU Counts (CPM)")
plt.xlabel("Values")
plt.ylabel("Frequency")

# Show the plot
plt.show()
```

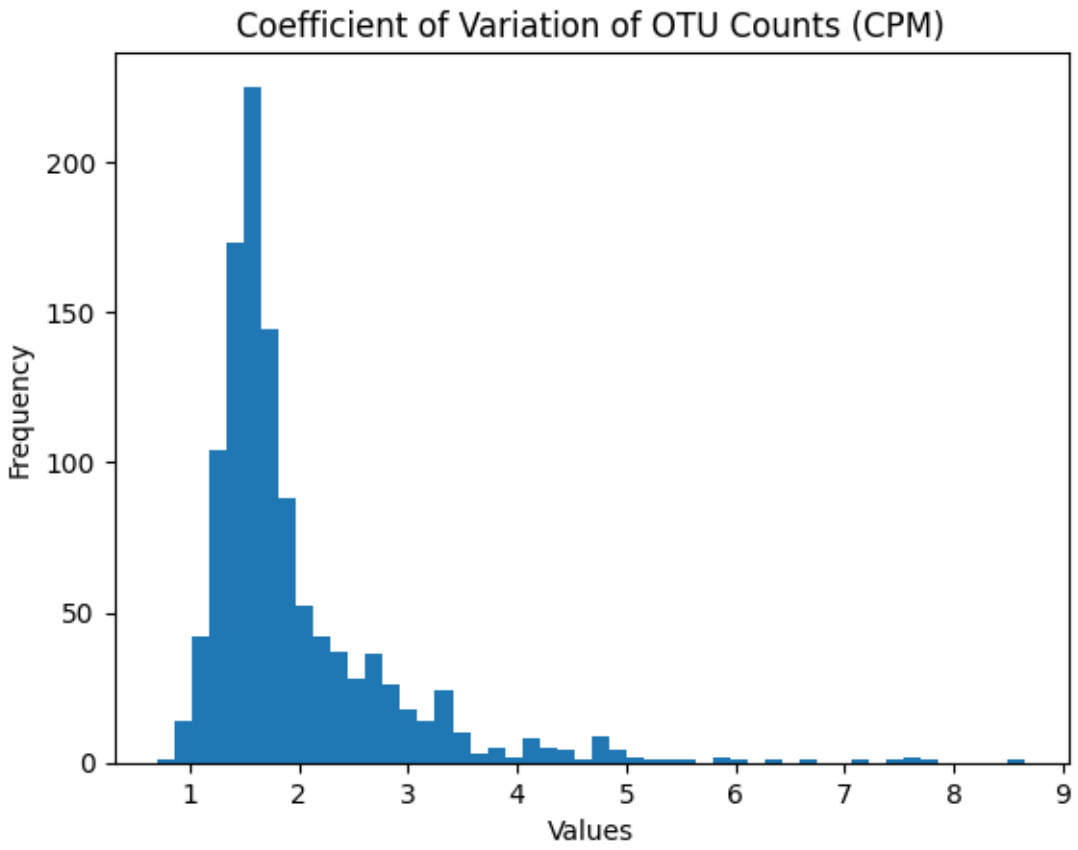


[ ]:
```
import matplotlib.pyplot as plt

otu_table_merged_night_zeros_filtered_cv.plot.hist(bins=50)

# Set the title and labels
plt.title("Coefficient of Variation of OTU Counts (CPM)")
plt.xlabel("Values")
plt.ylabel("Frequency")

# Show the plot
plt.show()
```



[ ]:
```
# Calculate the coefficient of variation for each row
otu_table_merged_day_zeros_filtered_cv2 = otu_table_merged_day_zeros_filtered.
 ↪loc[otu_table_merged_day_zeros_filtered_cv[otu_table_merged_day_zeros_filtered_cv␣
 ↪> 2].index]
otu_table_merged_night_zeros_filtered_cv2 =␣
 ↪otu_table_merged_night_zeros_filtered.
 ↪loc[otu_table_merged_night_zeros_filtered_cv[otu_table_merged_night_zeros_filtered_cv␣
 ↪> 2].index]
```

```
print(otu_table_merged_day_zeros_filtered_cv2.shape)
print(otu_table_merged_night_zeros_filtered_cv2.shape)
```

```
(334, 173)
(340, 172)
```

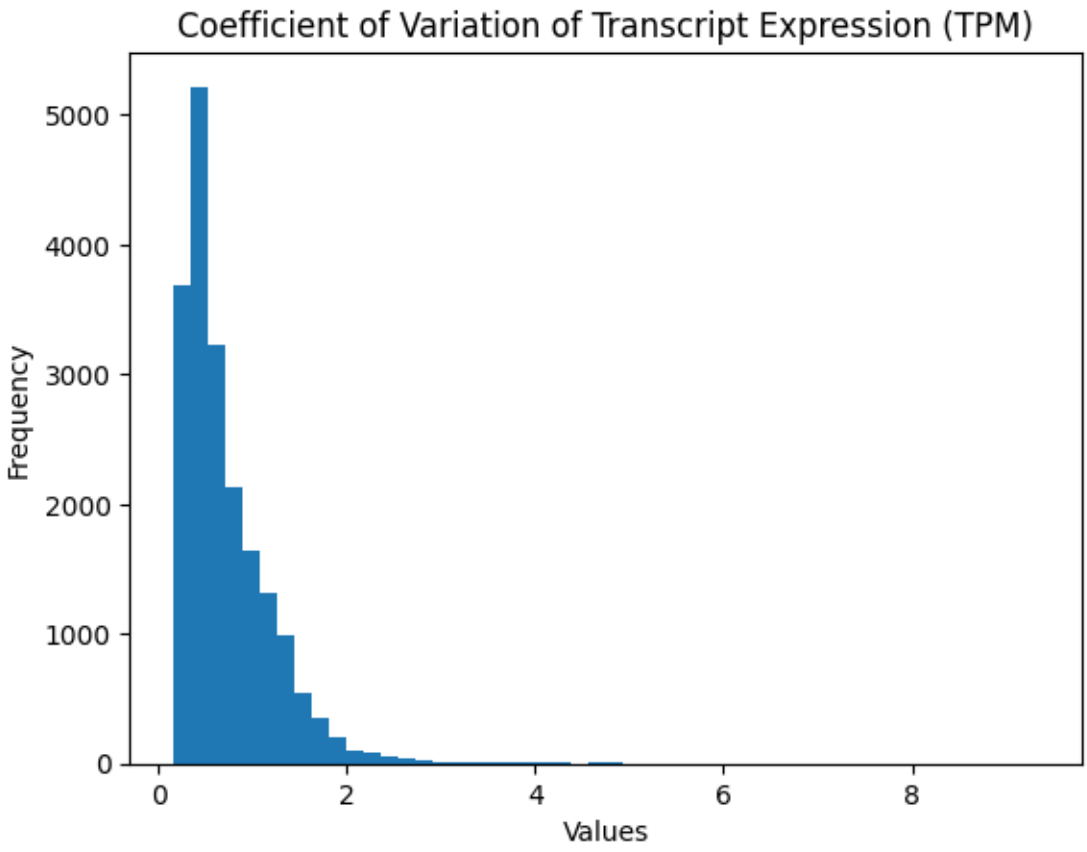
[ ]:
```
# Calculate the coefficient of variation for each row
kremling_expression_v5_day_renamed_zeros_filtered_cv =␣
 ↪kremling_expression_v5_day_renamed_zeros_filtered.apply(lambda row: np.
 ↪std(row) / np.mean(row), axis=1)
kremling_expression_v5_night_renamed_zeros_filtered_cv =␣
 ↪kremling_expression_v5_night_renamed_zeros_filtered.apply(lambda row: np.
 ↪std(row) / np.mean(row), axis=1)
```

[ ]:
```
import matplotlib.pyplot as plt

kremling_expression_v5_day_renamed_zeros_filtered_cv.plot.hist(bins=50)

# Set the title and labels
plt.title("Coefficient of Variation of Transcript Expression (TPM)")
plt.xlabel("Values")
plt.ylabel("Frequency")

# Show the plot
plt.show()
```

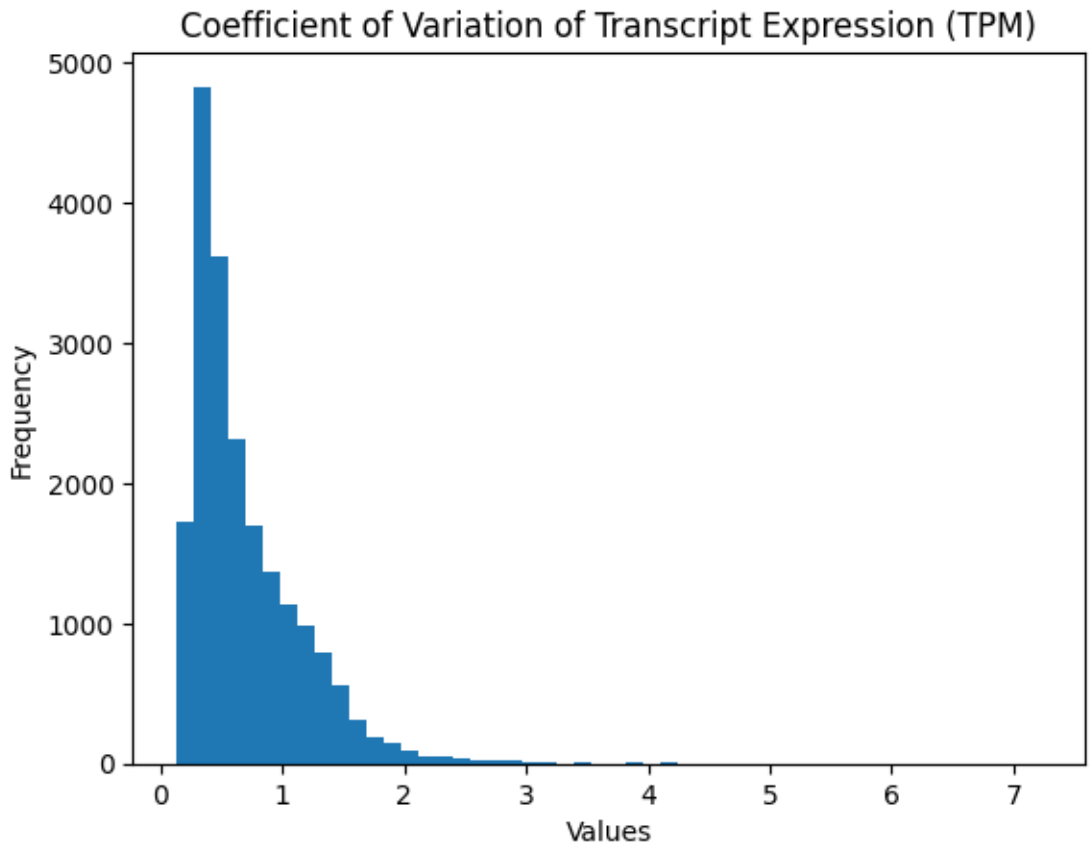


[ ]:
```
import matplotlib.pyplot as plt

kremling_expression_v5_night_renamed_zeros_filtered_cv.plot.hist(bins=50)

# Set the title and labels
plt.title("Coefficient of Variation of Transcript Expression (TPM)")
plt.xlabel("Values")
plt.ylabel("Frequency")

# Show the plot
plt.show()
```



[ ]:
```
kremling_expression_v5_day_renamed_zeros_filtered_cv1 =␣
 ↪kremling_expression_v5_day_renamed_zeros_filtered.
 ↪loc[kremling_expression_v5_day_renamed_zeros_filtered_cv[kremling_expression_v5_day_renamed
 ↪> 1].index]
kremling_expression_v5_night_renamed_zeros_filtered_cv1 =␣
 ↪kremling_expression_v5_night_renamed_zeros_filtered.
 ↪loc[kremling_expression_v5_night_renamed_zeros_filtered_cv[kremling_expression_v5_night_ren
 ↪> 1].index]
print(kremling_expression_v5_day_renamed_zeros_filtered_cv1.shape)
print(kremling_expression_v5_night_renamed_zeros_filtered_cv1.shape)
```

```
(4426, 173)
(4330, 172)
```

[ ]:
```
concatenated_day_df = pd.
 ↪concat([kremling_expression_v5_day_renamed_zeros_filtered_cv1,
                            otu_table_merged_day_zeros_filtered_cv2], axis=0)
concatenated_night_df = pd.
 ↪concat([kremling_expression_v5_night_renamed_zeros_filtered_cv1,
                            otu_table_merged_night_zeros_filtered_cv2], axis=0)
```

```
concatenated_day_transposed = concatenated_day_df.T
concatenated_night_transposed = concatenated_night_df.T
print(concatenated_day_transposed.shape)
print(concatenated_night_transposed.shape)
```

```
(173, 4760)
(172, 4670)
```

[ ]:
```
from corals.threads import set_threads_for_external_libraries
set_threads_for_external_libraries(n_threads=1)
import numpy as np
from corals.correlation.full.default import cor_full

cor_d_values = cor_full(concatenated_day_transposed)
cor_d_values.shape

highly_correlated_pairs_d_df = pd.DataFrame(columns=['feature1', 'feature2',␣
 ↪'correlation'])
correlated_pairs_file = open('correlated_pairs_merged_otus_day_transcripts.
 ↪txt', 'w')

# Find the highly correlated pairs
for i in range(len(cor_d_values.columns)):
    for j in range(i+1, len(cor_d_values.columns)):
        if cor_d_values.columns[i] == cor_d_values.columns[j] or\
            (str(cor_d_values.columns[j]).startswith('Zm') and str(cor_d_values.
 ↪columns[i]).startswith('Zm')):
            continue
        if ((abs(cor_d_values.iloc[i, j]) > 0.6) or\
           (abs(cor_d_values.iloc[i, j]) < -0.6)) and\
            (str(cor_d_values.columns[j]).startswith('Zm') or str(cor_d_values.
 ↪columns[i]).startswith('Zm')):
            pair = (cor_d_values.columns[i], cor_d_values.columns[j])
            highly_correlated_pairs_d_df.loc[len(highly_correlated_pairs_d_df.
 ↪index)] = [cor_d_values.columns[i],
 ↪                                                                          ␣
 ↪     cor_d_values.columns[j],
 ↪                                                                          ␣
 ↪     cor_d_values.iloc[i, j]]
            correlated_pairs_file.write(f'{cor_d_values.
 ↪columns[i]}\t{cor_d_values.columns[j]}\t{cor_d_values.iloc[i, j]}\n')

correlated_pairs_file.close()

cor_n_values = cor_full(concatenated_night_transposed)
```

```
cor_n_values.shape

highly_correlated_pairs_n_df = pd.DataFrame(columns=['feature1', 'feature2',␣
 ↪'correlation'])
correlated_pairs_file = open('correlated_pairs_merged_otus_night_transcripts.
 ↪txt', 'w')

# Find the highly correlated pairs
for i in range(len(cor_n_values.columns)):
    for j in range(i+1, len(cor_n_values.columns)):
        if cor_n_values.columns[i] == cor_n_values.columns[j] or\
            (str(cor_n_values.columns[j]).startswith('Zm') and str(cor_n_values.
 ↪columns[i]).startswith('Zm')):
            continue
        if ((abs(cor_n_values.iloc[i, j]) > 0.6) or\
           (abs(cor_n_values.iloc[i, j]) < -0.6)) and\
            (str(cor_n_values.columns[j]).startswith('Zm') or str(cor_n_values.
 ↪columns[i]).startswith('Zm')):
            pair = (cor_n_values.columns[i], cor_n_values.columns[j])
            highly_correlated_pairs_n_df.loc[len(highly_correlated_pairs_n_df.
 ↪index)] = [cor_n_values.columns[i],
                                                                              ␣
 ↪     cor_n_values.columns[j],
                                                                              ␣
 ↪     cor_n_values.iloc[i, j]]
            correlated_pairs_file.write(f'{cor_n_values.
 ↪columns[i]}\t{cor_n_values.columns[j]}\t{cor_n_values.iloc[i, j]}\n')

correlated_pairs_file.close()
```

```
/home/rsantos/Repositories/maize_microbiome_transcriptomics/.venv/lib/python3.10
/site-packages/corals/threads.py:48: UserWarning: This function should be called
before `numpy` or similar modules are imported.
  warnings.warn("This function should be called before `numpy` or similar
modules are imported.")
```

[ ]:
```
import pandas as pd
import matplotlib.pyplot as plt

grouped = df.groupby([pd.cut(df['values'], ranges), 'class']).size().unstack()

# Plot the histogram for each class
grouped.plot(kind='bar', stacked=True)

# Set the labels and title
plt.xlabel('Value Ranges')
```

```
plt.ylabel('Count')
plt.title('Histogram of Values by Class')

# Show the plot
plt.show()
```

[ ]:

	feature1	feature2	correlation
0	Zm00001eb368660_T001	New.ReferenceOTU1486	0.613635
1	Zm00001eb366860_T002	New.ReferenceOTU3269	0.741192
2	Zm00001eb363720_T002	928829	0.679813
3	Zm00001eb363720_T002	822187	0.662187
4	Zm00001eb358290_T002	808437	0.636430